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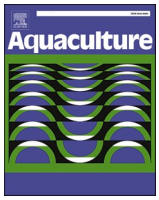

Effects of spawning success and rearing-environment on genome-wide variation of red drum in a large stock-enhancement program

Shannon J. O'Leary [a,b,*], Christopher M. Hollenbeck [a,c], Robert R. Vega [d], Ashley N. Fincannon [d], David S. Portnoy [a]

[a] Marine Genomics Laboratory, Texas A&M University Corpus Christi, Department of Life Sciences, Corpus Christi, TX 78412, USA
[b] Department of Biological Science, Saint Anselm College, 100 Saint Anselm Drive, Manchester, NH 03102, USA
[c] Texas A&M AgriLife Research, 600 John Kimbrough Boulevard, College Station, TX 78412, USA
[d] CCA Marine Development Center, Texas Parks and Wildlife, 4300 Waldron Road, Corpus Christi, TX 78418, USA

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ABSTRACT

Stock enhancement is an increasingly popular fisheries management strategy in which high mortality of early life history stages is circumvented by rearing juveniles in controlled or semi-controlled conditions. While the success of such programs is generally measured by the number of stocked individuals or individuals recruiting to the wild populations, an important consideration is minimizing impacts to the genetic diversity of wild populations by maximizing the number of breeders contributing to stocked progeny. The contribution of individual breeders is impacted both by variance in spawning success among adults and variance in mortality among families during grow-out. To disentangle the effects that these sources of variance have on parental contribution, red drum broodfish participating in three spawning events at the TWPD Marine Development Center were genotyped at several thousand SNP-containing loci along with their progeny. Progeny were sampled at three time points; shortly after introduction to grow-out ponds (T1), approximately midway through grow-out (T2), and as fingerlings during harvesting to be stocked in bays (T3). Using composite genotypes, progeny were assigned to parents to determine the reproductive success of individual broodfish at T1, T2, and T3 and to identify changes in the effective number of breeders (N_b) during rearing in grow-out ponds. Relationships between reproductive success and broodfish-specific parameters, including age and condition when introduced to the hatchery, time spent in the hatchery, and estimated age at spawning were assessed. Finally, associations between components of genomic variation in progeny from each sampling period and environmental parameters including salinity, pH, temperature, and dissolved oxygen present in the grow-out ponds, were identified. The results indicate that initial variance in reproductive success, including the failure of some individuals to spawn successfully, has the greatest impact on N_b. Mortality during growth had a relatively small impact on N_b and, in some cases, led to increases in N_b. Further, both family and environmental conditions in the outdoor rearing ponds significantly shaped the genetic diversity of stocked yearlings. Overall, these findings indicate that outdoor rearing in a semi-controlled environment exposes progeny to a range of environmental conditions across the season, which appears to play a role in maintaining and sometimes increasing N_b.

1. Introduction

Currently, only two-thirds of fish stocks are within biologically sustainable levels, highlighting the ongoing problem of collapsing fisheries due to overfishing, habitat fragmentation, and habitat loss (FAO, 2018). For coastal species with high mortality during larval recruitment, an increasingly popular strategy for mitigation is stock enhancement through the release of hatchery-reared juveniles into wild populations (Bell et al., 2006). While they share many similarities, aquaculture for the purposes of stock enhancement differs from commercial aquaculture in important ways. For commercial aquaculture, the goal is maximizing output (yield) by producing as many fast-growing individuals as possible using minimal resources. By contrast, stock enhancement programs' output and thereby success is measured by such metrics as the number of fingerlings stocked per season or the number of released hatchery individuals successfully recruiting to the fishery. Additionally, stock

* Corresponding author at: Marine Genomics Laboratory, Texas A&M University Corpus Christi, Department of Life Sciences, Corpus Christi, TX 78412, USA.
E-mail address: shannon.j.oleary@gmail.com (S.J. O'Leary).

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enhancement programs must engage in strategies aimed at minimizing negative impacts of stocking on the adaptive potential of wild populations (Blankenship and Leber, 1995).

One critical hatchery strategy to decrease adverse genetic impacts of stocking is maximizing the number of breeders contributing to cohorts released into the wild, quantified as the effective number of breeders (N_b; Blankenship and Leber, 1995). Supportive breeding programs can have negative effects on genetic diversity of wild populations if stocked individuals originate from too few broodfish because those broodfish may disproportionally contribute to a wild cohort (Ryman and Laikre, 1991). Even in a relatively large wild population (~1 mill) with modest levels of stock enhancement (approx. 10%), stocking could theoretically result in a reduction of effective population size and an accelerated loss of genetic diversity (Ryman and Laikre, 1991; Waples et al., 2016). Sex skew and variance in family size are key parameters leading to a reduction of N_b relative to the actual number of broodfish contributing to a spawning event (Hill, 1979). Thus, effective strategies to maximize N_b include regularly replacing existing broodstock with wild-caught adults and rotating existing broodstock among tanks to maximize mating combinations (McEachron et al., 1995). However, despite these efforts, it can be difficult to identify individual broodstock with low spawning success and minimize associated variance in reproductive success.

Reduction of N_b can occur across two stages of hatchery breeding: first, during spawning in tanks and subsequent transfer of eggs to the incubator, and second, during grow-out. Factors prior to grow-out that could impact family representation include individual broodstock fitness, viability of specific crosses, variation in egg quality, and variation in the effects of transport stress (Cason and Anderson, 2015). The grow-out stage, an essential component of hatchery programs, allows for a reduction in the normally high rate of mortality at larval and early juvenile stages, due to a lack of predators and ample supply of prey (Lorenzen, 2005). Because grow-out facilities are designed to create ideal conditions to maximize the number of individuals that survive to stocking by controlling nutrition, density of animals, salinity, temperature, dissolved oxygen, and pH (Cason and Anderson, 2015; McEachron et al., 1995), individuals with specific genetically influenced traits could be favored, leading to over/underrepresentation of families (domestication effect). Domestication effects may occur despite high levels of genetic diversity being maintained and can lead to loss of critical adaptive characteristics, making it difficult for stocked individuals to survive and reproduce in the wild. Rearing progeny in outdoor ponds in which conditions are not strictly controlled is not only economically more efficient but also may have the benefit of preserving a range of tolerance to abiotic conditions encountered by wild populations, thus maintaining key adaptive characteristics (Cason and Anderson, 2015; Feuerbacher et al., 2016; Tave and Hutson, 2019). Understanding the relative impact of the initial variance in reproductive success and mortality during grow-out on the number of contributing breeders is important when the goal is to avoid negatively impacting the adaptive potential of wild populations (Ryman and Laikre, 1991; Waples et al., 2016).

The Texas Parks and Wildlife (TPWD) Coastal Hatcheries Program has augmented the natural population of red drum since 1983 by releasing 15–30 million fingerlings annually into eight bays and estuaries (Vega et al., 2003), and has implemented a number of strategies to safeguard natural genetic diversity. Because of red drum size and spawning behavior, spawning tanks are limited to five individuals; however, 25% of broodstock are replaced annually with new breeders captured in the wild. Additionally, existing broodstock are regularly rotated among tanks to maximize mating combinations (McEachron et al., 1995). Finally, larvae are reared in outdoor grow-out ponds where physical parameters (temperature, dissolved oxygen, pH, salinity) are monitored but allowed to vary before being released as fingerlings into the bays. Despite these practices, previous research using microsatellites has indicated variance in reproductive success mainly attributable to the spawning process with additional variance attributable to grow-out, though this effect is lower in magnitude and much less consistent (Anderson et al., 2017; Gold et al., 2008).

Disentangling whether variance in spawning and reproductive success are random effects or attributable to specific traits that result in higher reproductive success in hatchery conditions is important for informing hatchery procedures. In addition, understanding how variation in abiotic and biotic conditions during the rearing process may result in non-random differences in survival is important for informing procedures aimed at maintaining genetic diversity. However, because selection acting on a given trait may only influence a small proportion of the genome, microsatellite loci lack the resolution to address these questions. Furthermore, once informative SNPs have been identified, genotyping-in-thousands by sequencing (GTseq)-panels can be developed for long-term monitoring, which are quickly becoming more cost and time-efficient as compared to microsatellite panels and can encompass sex-specific loci, loci putatively under selection, in addition to those being used for parentage analysis (Campbell et al., 2015). Therefore, broodstock at the TPWD Marine Development Center (MDC) and their progeny were genotyped at thousands of single nucleotide polymorphisms (SNPs) across both coding and non-coding regions throughout the entire genome, allowing for the simultaneous identification of sibling groups and parent-offspring relationships and assessment of correlations between components of genomic variation and environmental conditions. Progeny were sampled at the beginning, middle, and end of their time in the grow-out ponds and assigned back to parents to assess reproductive success during the early life stages to identify non-random changes in family representation occurring during grow out. The goals of the study were to (1) disentangle the impact of variance in reproductive success of adults and the variance caused by mortality of progeny during grow-out, (2) compare variance in reproductive success with conditioning parameters measured in brooders, and (3) test for a correlation between environmental conditions in grow-out ponds and genetic variation of progeny.

2. Materials and methods

2.1. Spawning and sampling design

Spawning tanks at the MDC typically contain three females and two males each. The facility houses a total of three rooms with four tanks each (60 broodstock); yearly, broodstock are rotated among the tanks within and among rooms with 25% of the broodstock replaced by wild-caught individuals to maximize effective number of breeders and potential pairings across years. No broodstock are selected from within the hatchery, thus avoiding potential inbreeding at this stage. After spawning occurs in at least one tank, eggs are collected and transferred to an incubator for volumetric enumeration to quantify egg density. Up to 1.2 million eggs are then stocked into 100-gal incubators on a flow-through system. If adults from more than one tank spawn on the same night, incubators contain eggs that represent all spawning tanks. After two days in the incubator room, larvae are enumerated and stocked into grow-out ponds at a rate of 400,000 larvae per acre. For this project, three grow-out ponds stocked with individuals from separate spawning events from the same set of spawning tanks in a single room were sampled in 2017 (Spawning Event 1, stocked 10/10/2017) and 2018 (Spawning Event 2 and 3, stocked 9/22/2018 and 9/27/2018, respectively) at three time points. Approximately 100 individuals were removed using a dip net at approximately 10 days after stocking (T1), midway through grow-out (T2), and as fingerlings prior to stocking in local bays (T3). Broodstock contributing to Spawning Events 1, 2, and 3 came from two, four, and three tanks, respectively (Table 1); due to the broodstock rotations only a subset of broodstock were involved in more than one spawning event across years.

Table 1
Total number of male and female broodfish per Spawning Event in spawning tanks, the expected proportion of progeny assigned per individual if all contribute equally and the number of broodfish that did not have progeny assigned to them.

Spawning event	$N_{(tanks)}$	Sex	$N_{(indv)}$	Expected progeny (%)	Unsuccessful
1	2	F	5	20	2
		M	5	20	0
2	4	F	11	9.09	6
		M	9	11.11	2
3	3	F	9	11.11	2
		M	6	16.67	1

2.2. Genotyping and parentage assignment

Fin clips from all broodfish ($N = 21$) and whole progeny ($N = 1085$) were preserved in salt-saturated 20% DMSO buffer. DNA was extracted using Mag-Bind Blood and Tissue DNA kits (Omega Bio-Tek). Double digest restriction-site associated DNA (ddRAD) libraries were constructed using a modified protocol (Portnoy et al., 2015) and sequenced on seven lanes of an Illumina HiSeq 2500. Reads were demultiplexed using *process radtags* (Catchen et al., 2011), low quality individuals with <50,000 reads were removed and quality-filtered reads mapped to a draft red drum reference genome (unpublished), and SNPs called using the *dDocent* pipeline (Puritz et al., 2014). Separate data sets were created for parentage assignment and analysis of correlation of genetic variation in progeny to environmental conditions in the grow-out ponds. Briefly, each raw SNP data set was filtered using *vcftools* (Danecek et al., 2011) and custom scripts, according to principles set forth in O'Leary et al. (2018). The final threshold values for loci included in the data set were a genotype quality >30 and locus quality >20, minimum 10 reads per genotype, a minimum mean depth of ≥20 for a locus across all individuals, and a genotype call rate > 90%. Individuals with >50% missing data were excluded from further analysis. Replicate individuals sequenced on different runs and comparisons of patterns of expected and observed homozygote and heterozygote calls were used to assess genotyping error (Anderson, 2018). For parentage assignment, several hundred reliably scored (low genotyping error), independent, and informative loci (high minor allele frequency/high genotyping call rate) were used to ensure a sufficient number of loci for full pedigree reconstruction (Huisman, 2017). Therefore, the data were additionally filtered to remove one SNP from each pair of SNPs with high linkage disequilibrium ($r^2 > 0.5$) and SNPs with a minor allele frequency < 1% (individual filtering steps are detailed in Supplementary Material 2).

Parentage assignment was performed using *Sequoia* (Huisman, 2017). Sample information, including sex and birth year, was used to differentiate among parents and offspring in the program; therefore, all parents had their birth year arbitrarily set to 2010 (before birth years of progeny), while progeny had birth years in 2017 and 2018. Full pedigree reconstruction involved an initial sibship clustering among individuals followed by a reduction of parent-offspring pairs using opposing homozygotes, then in consecutive rounds, likelihoods of all possible relationships were compared until the total likelihood converged. *Sequoia* was run with an assumed genotyping error rate of 0.02, a safety margin for the allowed number of opposite homozygotes of 10, and threshold log10-likelihood ratios (LLR) set to the default values.

2.3. Comparison of effective number of breeders

The maximum N_b attainable (i.e., all broodfish contributing with equal success) given the intentional skew in sex ratio of broodfish at the hatchery was calculated as:

$$N_{b(max)} = \frac{4(N_f N_m)}{N_f + N_m} \quad (1)$$

with N_f and N_m denoting the number of females and males potentially contributing to a spawning event (i.e., present in the spawning tanks), respectively.

N_b was calculated at each sampling point within each spawning event to examine changes relative to the census number of broodstock in the tanks (N) due to variance in family size and rearing, as

$$N_b = \frac{4(N_{bf} N_{bm})}{N_{bf} + N_{bm}} \quad (2)$$

following Crow and Kimura (Crow and Kimura, 1971), with the effective number of females N_{bf} calculated as

$$N_{bf} = \frac{1}{\sum_{k=1}^{n_f} q_k^2} \quad (3.1)$$

and the effective number of males N_{bm} calculated as

$$N_{bm} = \frac{1}{\sum_{k=1}^{n_m} q_k^2} \quad (3.2)$$

where q is the proportion of progeny contributed by each female or male (Lacy, 1989).

2.4. Comparison of expected and realized reproductive success and condition parameters

For each spawning event, the realized reproductive success of a broodfish for T1, T2, and T3 was calculated as the proportion of progeny assigned to that individual, while expected reproductive success was determined as the proportion of progeny that would be assigned to a given broodfish if every individual of the same sex contributed equally. Changes in reproductive success over time and differences between expected and realized reproductive success of individual broodfish were assessed within and among spawning events.

For all broodfish, length, weight, and year when they were introduced to the hatchery were noted. Condition at introduction was calculated as the weight/length ratio. The approximate age at introduction was calculated using an age-length relationship reported by (Porch et al., 2002). Approximate age at spawning was determined as the sum of age at introduction and time spent in the hatchery. Linear regressions were used to test for significant relationships between reproductive success of broodfish at T1, T2, T3, and condition parameters. For breeders participating in multiple spawning events, reproductive success in different events was treated independently. In addition, individuals participating in more than one spawning event were classified into three categories, "always successful", "sometimes successful", and "never successful". Then differences in condition parameters associated with those categories were identified using a Kruskal-Wallis test,a non-parametric test that compares mean ranks (medians) of groups and is less sensitive to outliers than tests using means. Significant pairwise differences were determined post-hoc using a Dunn's test and corrected for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR) method (5% cut-off). Additionally, the Spearman's rank correlation coefficient was calculated to determine the significance of correlation between conditioning parameters and reproductive success at T1 and T3.

2.5. Correlations of progeny genotypes and environmental parameters in grow-out ponds

Physical parameters (temperature, dissolved oxygen, pH, salinity) in grow-out ponds are monitored by TPWD. Apart from dissolved oxygen, which can be manipulated to maintain desired levels, parameters are not controlled. Rather, they reflect natural environmental heterogeneity, i.e., conditions progeny would encounter in the estuaries during that time period. Heterogeneity of physical parameters among grow-out ponds was tested using ANOVA, and post-hoc Tukey's Honest Significant

Difference tests were used to explore differences in a pairwise manner. Grow-out ponds exclude predators and are regularly fertilized to ensure an abundance of prey (zooplankton).

A partial redundancy analysis (RDA) is a constrained ordination method used to determine the extent to which one set of constraining variables (environmental data) explains the variation in another set of response variables (allele frequencies), while controlling for a set of conditioning variables that might otherwise obscure the effects of the constraining variables (family). A partial RDA was implemented in *vegan* (Oksanen et al., 2013) to identify alleles that are correlated with the environment. Constraining variables (mean, 5th, 50th, and 95th quantile) were calculated for each of the three sampling time points for a given abiotic factor (i.e., temperature, dissolved oxygen, pH, and salinity) from the day progeny were stocked to time points T1, T2, and T3, respectively. Variables for the model were selected using stepwise selection and forward selection of variables; variables selected by both models were retained. Significance was tested using a permutation test (1000 permutations) as implemented in *vegan*.

Variance partitioning was used to compare the contribution of family and environmental parameters in structuring the observed genomic variation. A full model using family and environmental variables, and partial models using family conditioned on environment and environmental variables conditioned on family were considered to partition the explainable variance into individual (family, environment) and shared components (family + environment) using *vegan*. Significance of each component was tested using 1000 permutations. Finally, a Mahalanobis distance >13.8 was used to identify alleles with the strongest association to the first two RDA axes (Capblancq et al., 2018; Forester et al., 2018).

Unless otherwise noted, analyses were performed in R (R Core Team, 2013) using cited packages, and figures were generated using *ggplot2* (Wickham, 2009). Extended, fully reproducible methods documenting details of bioinformatic processing steps and statistical analysis are available as Supplementary materials 1 – 5 (Rmarkdown/html-documents) and as a GitHub repository at https://github.com/sjoleary/SOC_ParAssignm.

3. Results

3.1. Spawning

The number of eggs placed in the incubator after each spawning event was similar for Spawning Events 1 and 3 (approx. 1 million) in contrast to Spawning Event 2, where approximately 690,000 eggs were placed in the incubator, despite the large number of broodfish (four tanks) involved in Event 2. Though larvae were stocked into the grow-out ponds at similar levels (375,000–428,000), the proportion of progeny that survived to be harvested for stock enhancement varied greatly from 7% (Spawning Event 3) to 28% (Spawning Event 1), and 91% (Spawning Event 2). By contrast, survival among spawning events was largely consistent during the time in the incubator (52–60%). Despite Spawning Event 2 having the lowest number of eggs incubated, it had the highest number of larvae harvested (343,000) compared to Events 1 and 3 (111,000 and 30,000, respectively).

Young-of-the-year generally exhibit linear growth and are expected to spend approximately 30 days in a grow-out pond to reach a desired length of 2.5–3 cm, at which time they are harvested. Linear regressions of progeny size and time spent in grow-out ponds showed similar slopes for Spawning Events 1 (0.3) and 2 (0.34), while the slope for Spawning Event 3 was lower (0.1; see Supplementary Material 5, Fig. 1 for details). Fish were harvested from the grow-out pond for Spawning Event 1 at a shorter size (1.5 cm) than usual due to a facility construction project on the pond levees. Due to slow growth from low pond temperatures, fish from Spawning Event 3 were also harvested at a smaller size (1.2 cm, compared to 2.4 cm for Event 2), but spent the longest time in the grow-out ponds (64 days, compared to 36 and 48 days for Spawning Event 1 and 2, respectively).

3.2. Genotyping and parentage assignment

The filtered SNP data set used for parentage assignment consisted of 800 loci. Genotyping error estimated across individuals sequenced in replicate was <5% across all loci. The overall mean genotyping error assessed using patterns of observed and expected homozygous and heterozygous calls was <0.001%. Two adults (777, 5081) were assigned as full siblings, though due to the way *Sequoia* distinguishes full siblings from parents and their estimated ages, they could potentially be parent-offspring. Further, exploratory analysis indicated that one broodfish participating in spawning event 1 and 2 originally designated as female was male; all calculations of expected reproductive success and number of male/female broodfish participating in a spawning event reflect the true male/female ratios in the tanks. Twelve progeny were removed from the data set due to the ambiguity of parentage assignment. The final pedigree consisted of 830 progeny unambiguously assigned to seven different females and ten different males.

3.3. Comparison of effective number of breeders

For spawning events 2 and 3, $N_{b(max)}$ was marginally smaller than the number of broodfish present in the spawning tanks as expected due to the fact that generally spawning tanks contain three females and two males. Due to the misidentification of the sex of one individual for Spawning Event 1, $N_{b(max)}$ was equal to the number of individuals in the contributing tanks. However, because in each event some individuals did not contribute to a given sample, i.e. none of their offspring were detected in the sample, the maximum attainable effective number of breeders assuming no variance in reproductive success of only those individuals that contributed ($N_b{}'_{(max)}$) was 25–41% smaller than $N_{b(max)}$. Overall, variance in family size, caused by some individuals not contributing and uneven reproductive success among those who did, reduced N_b by 40–80% compared to $N_{b(max)}$. For Spawning Event 1, N_b increased from 5.56 (T1) to 5.95 (T3), while for Spawning Event 2, there was a slight decrease from 8.82 (T1) to 7.59 (T3). Spawning event 3 experienced the largest shift, with N_b increasing from 2.84 (T1) to 5.28 (T3) between sampling points (Table 2).

3.4. Comparison of expected and realized reproductive success and condition parameters

The expected reproductive success varied from 9%, for females in Spawning Event 2, to 20%, for both males and females in Spawning Event 1 (Table 1). However, parental assignment indicated that only a subset of broodfish present in tanks successfully contributed to a given spawning event (Fig. 1), and there was considerable variation in family size among spawning pairs (Fig. 2). For broodfish that contributed to a spawning event, realized reproductive success varied from 1% (3160 in Spawning Event 3 at T2) to 83.3% (4136 and 7930 in Spawning Event 3 at T1). There were also differences in reproductive success of individual broodfish across sampling points: the largest decrease was 31% (4136 in Spawning Event 3, from T1 to T3) and the largest increase was 23% (4134 in Spawning Event 3, from T1 to T3; Fig. 1). Male broodfish successfully spawned with an average of 1.3 to 1.4 females and female broodfish with an average of 1.8 to 2.3 males. The proportion of progeny in a full sibling family at harvest varied from 2.4% to 31.5% for Spawning Event 1, 1.7% to 38.5% for Spawning Event 2, and 1% to 52.6% for Spawning Event 3 (Fig. 2). In general, at T1, the difference between expected and realized reproductive success had more extreme values (positive and negative), and this effect usually decreased over time. Nevertheless, at T3, several males and females generally had higher than expected reproductive success, with the remaining broodfish having only marginally more or less progeny than expected. Spawning Event 3 stood out; here, a single male and female had 67 and 72% more progeny than expected assigned to them, respectively, at T1, which decreased to approximately 40% at T3 (Fig. 3).

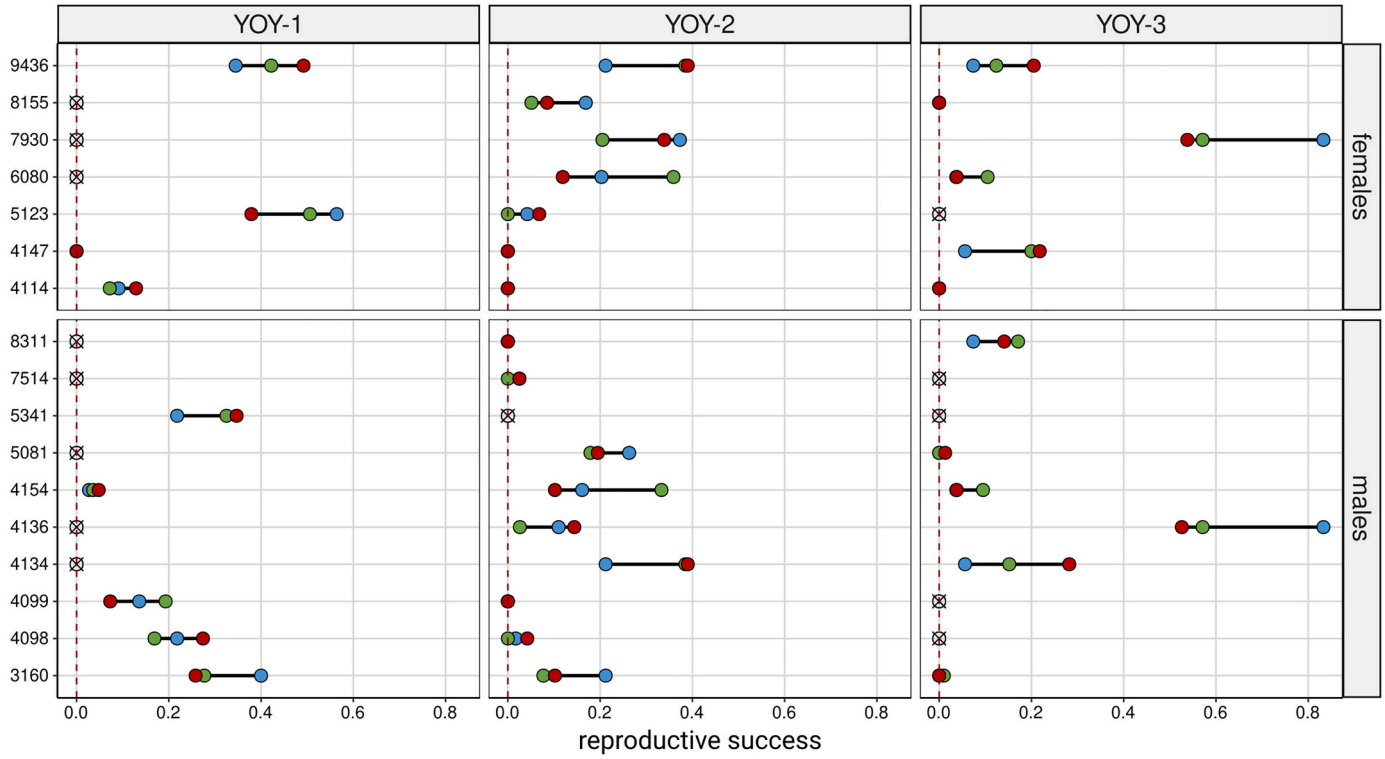

Fig. 1. Proportion of progeny assigned to each broodfish per Spawning Event (YOY 1–3) at each sampling point (T1, blue; T2 green; T3, red). Broodstock that were not in the spawning tanks for a given Spawning Event are denoted with an ⊗. The red dashed line indicates zero. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Comparison of actual and effective number of breeders per Spawning Event and sample point. N_{tanks} (N_M/N_F) indicates the number of males and females in all spawning tanks contributing to a Spawning Event, N_b(max) indicates the maximum attainable effective number of breeders if all broodfish contribute and contribute equally. $N_{contrib}$ indicates the number of adults that had progeny assigned to them, with $N'_{b(max)}$ (reduction) indicating the maximum attainable effective number of breeders given no variance in reproductive success among those adults contributing to a Spawning Event and the percent reduction compared to $N_{b(max)}$. Finally, N_b (reduction) indicates the effective number of breeders per sampling point and Spawning Event accounting for variance in reproductive success and the % reduction compared to $N_{b(max)}$.

Spawning event/ Sample point		N_{tanks} (N_M/N_F)	$N_{b(max)}$	$N_{contrib}$	$N'_{b(max)}$ (reduction)	N_b (reduction)
1	T1	10	10.0	8	7.5 (25%)	5.56 (44%)
	T2	(5/5)				5.81 (42%)
	T3					5.95 (40%)
2	T1	20	19.8	12	11.7 (41%)	8.82 (55%)
	T2	(9/11)				6.46 (67%)
	T3					7.59 (62%)
3	T1	15	14.4	9	8.9 (38%)	2.84 (80%)
	T2	(6/9)				5.13 (64%)
	T3					5.28 (63%)

Reproductive success at T1 and T3 were significantly correlated ($p = 0.87$ for females, $p = 0.98$ for males, $p < 0.001$), indicating that, with a few notable exceptions, individuals with initially high spawning success also exhibit high reproductive success at the time progeny were harvested for release into bays. No significant correlations were found between reproductive success and measured condition parameters of broodstock (Supplementary material 4, Figs. 15 and 16). Despite the observation that successful broodfish generally shared certain characteristics (Fig. 4), such as lower ages at spawning and date of introduction to the hatchery, as well as higher condition factor at introduction to the hatchery compared to broodfish that were never successful, Kruskal-Wallis tests were non-significant (p-value age at spawning = 0.259, p-value age introduced to hatchery = 0.277, p-value time spent in hatchery = 0.355).

3.5. Correlations of progeny genotypes and environmental parameters in grow-out ponds

The data set used to assess genetic-environmental correlation consisted of 2023 SNPs. Global tests of heterogeneity were significant for all environmental parameters measured in the grow-out ponds. Pairwise comparisons revealed significant differences in environmental conditions both among spawning events and among time points within spawning events. Following model selection procedures, the six selected parameters for the constraining matrix in the RDA consisted of the 95th quantile of pH, mean temperature for the afternoon, mean evening dissolved oxygen, 5th quantile of pH, and the 50th quantile of salinity. The RDA was significant ($p < 0.001$) and the adjusted R^2 value was 0.001. Variance partitioning indicated that the largest component of variance was explained by family, environmental parameters, and a shared effect (0.390; Table 3). While all components of variance were significant, the component explained by family alone was 0.310, which is two orders of magnitude larger than the component explained by environmental parameters alone (0.001; Table 3). Individuals clustered most tightly for Spawning Event 1; clusters were more distinct, though largely overlapping, for Spawning Event 2 and 3, which experienced a wider range of environmental parameters (Fig. 5). A total of 33 loci exhibited an allele with a Mahalanobis distance >13.82 (p-value <0.01); changes in allele frequencies over time across all individuals and by family can be viewed in Supplementary Material 5 Figs. 8a and 8b.

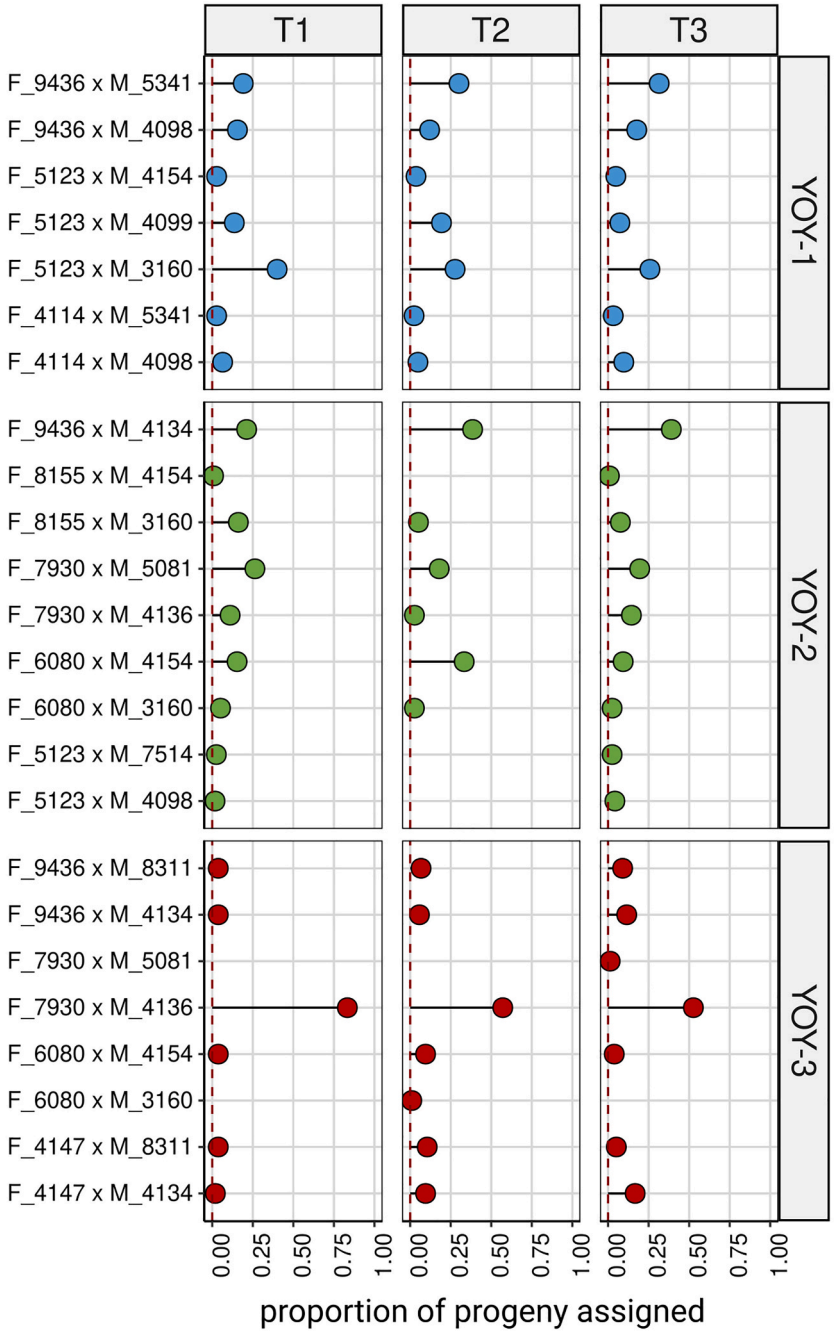

Fig. 2. Distribution of family size per Spawning Event (1, blue; 2, green; 3 red) and sampling point as proportion of total progeny assigned to each female x male cross. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

Here, red drum broodstock participating in three separate spawning events and their progeny, collected at multiple time points from outdoor grow-out ponds, were genotyped to assess changes in reproductive success among broodstock and identify potential factors that reduce N_b for individuals produced for stock enhancement. Overall, results show that declines in N_b, relative to the actual number of spawners, were primarily attributable to a subset of individuals in spawning tanks not participating in spawning and high variance in initial reproductive success among fish that spawned. During pond culture, N_b was found to decrease or increase, though the magnitude of change relative to the difference between the initial N_b at T1 and $N_{b(max)}$ was small. There were no significant correlations between reproductive success and condition parameters of adults, though adults that consistently failed to contribute shared certain characteristics. In addition, broodstock that failed to reproduce in one spawning event, generally had very low success in others they participated in. Partial RDA and variance partitioning indicated that both family and environmental conditions during outdoor rearing affect the genetic diversity of stocked progeny. Overall, these patterns further emphasize the importance of maximizing the number of mating combinations for each individual spawning event and within and across entire breeding seasons to maintain the genetic diversity of stocked individuals (García-Fernández et al., 2018) and points towards the importance of heterogeneous conditions found in grow-out ponds in maintaining that diversity, as progeny produced and stocked at different times across the spawning season experience different environmental conditions (Belk et al., 2008; Rasmussen et al., 2009).

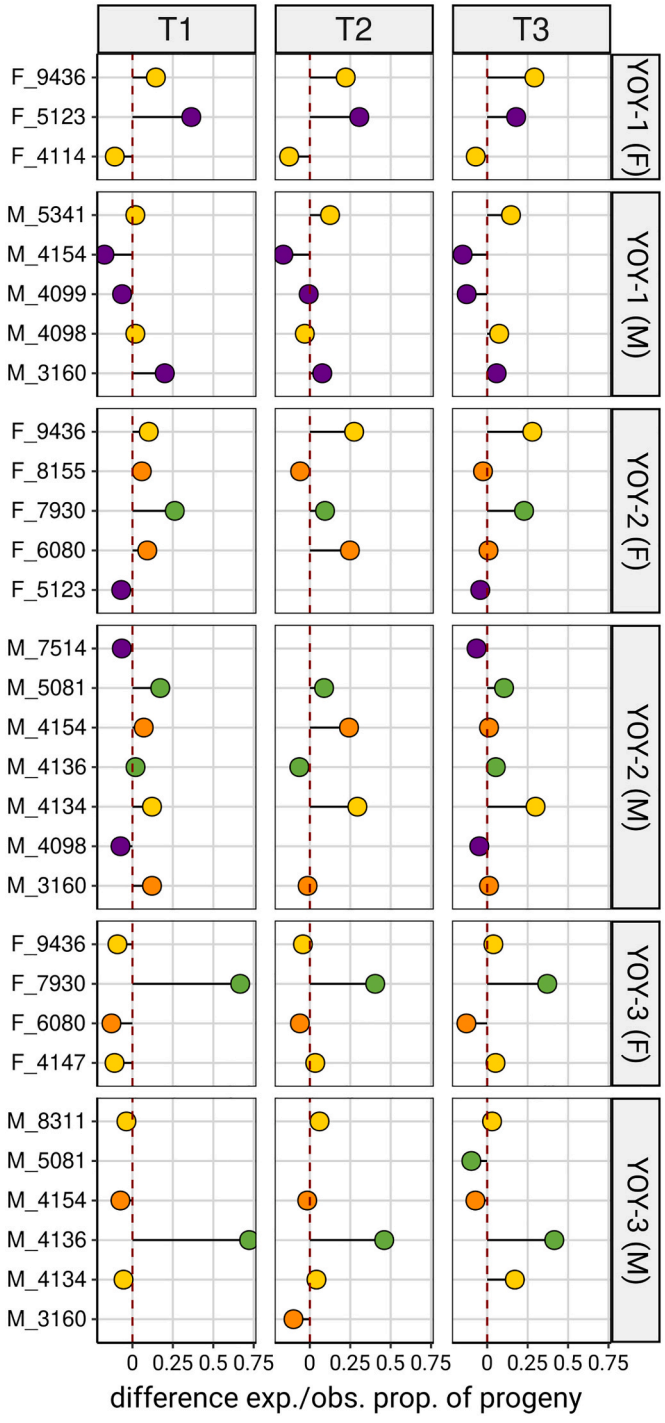

Fig. 3. Difference between expected and observed proportion of progeny per male and female per sampling point and Spawning Event. Breeders that did not have any progeny assigned are not included in figure. Colors correspond to broodfish' spawning tank (tank 4–1, orange; tank 4–2 green; tank 4–3 yellow; tank 4–4, purple). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The largest reductions in genetic diversity occurred because spawning events were dominated by a few individuals, with 2–6 individuals not participating in individual spawning events. While the condition parameters measured were not significant contributors to success, individuals that were introduced to the hatchery at a younger age and in better condition tended to be more successful. By contrast, individuals that did not contribute to spawning events generally had been introduced to the hatchery at an older age, spent more time in the hatchery, and tended to be female. In aquaculture settings where individual matings cannot be controlled but rather progeny are produced through batch spawning, it is common to observe that contributions from individual breeders will vary from event to event and will generally include a subset of individuals that do not contribute at all and that the level of success of individuals may vary across spawning events within a season (García-Fernández et al., 2018; Nugrohoa and Taniguchi, 2004; Perez-Enriquez et al., 2020). Additionally, significant relationships between characteristics of individual broodstock, such size and reproductive success, have been identified and can lead to individuals contributing disproportionally to individual spawning events or across entire spawning seasons (Smith et al., 2015).The observed differences in initial spawning success could be due to other factors such as unsynchronized spawning within tanks, resulting in progeny from some crosses having more time to deplete their yolk sac, sib-groups having an early growth or hatching advantage, incompatibilities, or maternal effects (Anderson et al., 2017). While it remains unclear why some animals failed to reproduce, the results here indicate that using genetic monitoring to identify and replace individuals that consistently fail to contribute to progeny is crucial for maximizing N_b and minimizing potential impacts on wild stocks. Finally, this highlights the importance of rotating broodstock across tanks, rooms, and regularly bringing in new broodstock to maximize genetic diversity. This is in line with findings from other parentage assignment studies that found it is better to use multiple smaller sets of broodstock and combine offspring across spawning events to increase the overall number of families obtained within and across breeding seasons to obtain more balanced parental contributions (García-Fernández et al., 2018; Nugrohoa and Taniguchi, 2004). Indeed, recent assessments of wild red drum populations in Texas bays that are stocked indicate that there is no long-term impact on the effective population size of wild populations (Hollenbeck et al., 2016).

Table 3
Partitioning of variance explained by family (fam), environmental variables (env), and shared effects due to correlation of family and environmental parameters in RDA model.

Partition	Variance	p-value
Residuals	0.610	n/a
fam + env + shared	0.390	0.001
fam + shared	0.389	0.001
fam	0.310	0.001
env + shared	0.080	0.001
shared	0.079	n/a
env	0.001	0.001

The ability to identify characteristics of individuals with consistently higher reproductive success in captivity is a double-edged sword. It allows for the use of individuals that could increase hatchery productivity but may result in unintentional selection of phenotypes that are successful under hatchery conditions but not in the wild. Fish culture practices honed for commercial production, including protection during spawning, regular feeding, water quality management, predator control, and health monitoring, may contribute to adaptation to hatchery conditions (domestication) that are counterproductive for stock augmentation (Doyle et al., 1995; Gilligan and Frankham, 2003; Tave and Hutson, 2019). In the production system studied here, the focus is on producing individuals that resemble wild fish by conserving genetic diversity, minimizing domestication effects, and avoiding negative impacts on wild populations. Domestication effects and loss of genetic diversity are almost unavoidable during hatchery-based stock augmentation even when broodfish are captured every generation and N_b is maximized (Finger et al., 2018; Lorenzen et al., 2012). Therefore, broodstock selection remains critical, with emphasis on selecting individuals that reflect the genetic and ecological diversity of wild populations (Le Vay et al., 2007).

While by far the largest reductions of N_b, compared to the maximum

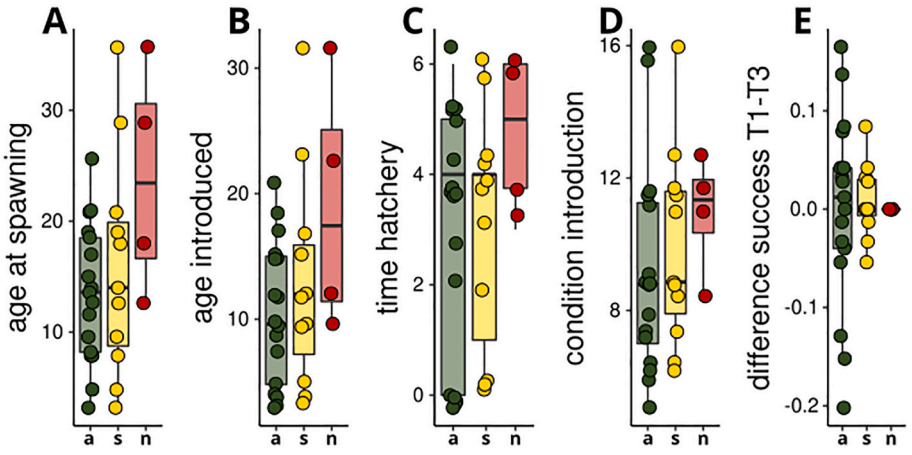

Fig. 4. Distribution of (A) age at spawning, (B) age at introduction, (C) time spent in the hatchery, and (D) difference in success from T1 to T2 for breeder groups as always successful (a, green), sometimes successful (s, orange), and never successful (n, red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

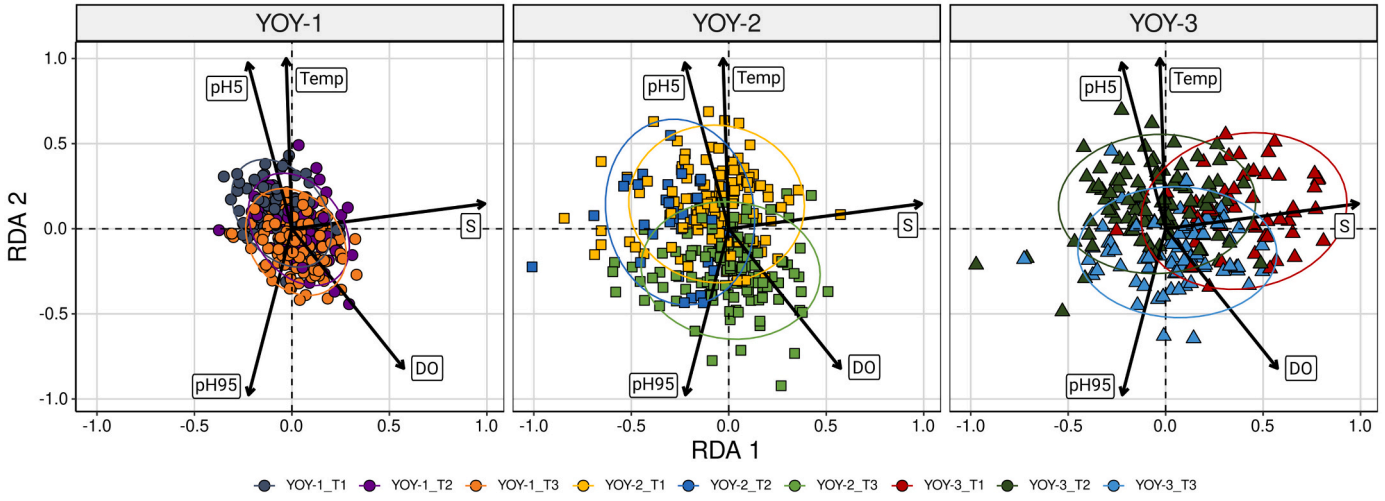

Fig. 5. Biplot of partial RDA showing clustering of individuals by Spawning Event 1–3 and sample point (fill) and environmental parameters (pH 5, pH 5th quantile; pH 95, pH 95th quantile; Temp, mean afternoon temperature; S, Salinity 50th quantile, DO, mean dissolved oxygen evening).

attainable N_b, were due to variance in spawning success, further changes of N_b occurred during grow-out. However, these changes were relatively minor and did not follow a consistent pattern. In Spawning Event 3, for example, a single group of full siblings accounted for 75% of the individuals sampled at T1 but at stocking represented approximately 50% of the individuals sampled. The result was an 80% reduction in N_b at the first sampling, relative to the maximum N_b possible ($N_{b(max)} = 14.4$, $N_{bT1} = 2.84$), followed by a small increase in N_b (5.28) at the time of stocking. This indicates that under certain circumstances, the grow-out stage may act as a buffer reducing variance in family representation and increasing the effective number of breeders represented in stocked fingerlings, an important consideration for reducing potential negative genetic impacts of stock augmentation (Hedrick et al., 2000; Lorenzen, 2005; Ryman and Laikre, 1991). These differences in the magnitude of reduction of N_b across T1-T3 indicate that differences in offspring viability, egg quality, egg provisioning, and/or stress tolerance might ultimately dictate survivorship (Anderson et al., 2017; Cason and Anderson, 2015). While previous studies on red drum survival in grow-out ponds demonstrated that much of the variation in survival rates could not adequately be explained by abiotic and biotic conditions alone, our results indicate that especially during environmental conditions that fall on the extremes of tolerable ranges, the genotypes of offspring may be an important factor determining survivorship.

The finding that components of genetic variation were significantly correlated to abiotic parameters in the grow-out ponds indicates that selective pressures imposed during pond grow-out may be responsible for changes in family representation over time. The idea is further supported by the observation that some individuals that participated in multiple events had higher reproductive success in some events as compared to others and abiotic conditions varied across those events (Fig. 1; e.g., female 7930, male 4136). Similarly, studies of June sucker (*Chasmistes liorus*) reared in hatchery and lake environments have demonstrated differences in morphology within families attributable to grow out environment as well as greater survival for fishes grown in lake conditions (Belk et al., 2008; Rasmussen et al., 2009), indicating that keeping fishes in "natural conditions" may be critical for stocking success. Other studies have indicated that captive stocks can rapidly diverge both genetically and morphologically from their wild counterparts, stressing the need for introduction of wild brooders to captive populations to ensure released individuals will persist in natural conditions (Black et al., 2017; Wilke et al., 2015). Given the limited marker-density, it is difficult to use the loci significantly associated with environmental conditions to determine which genes are under selection. Future work to fully annotate the red drum genome could result in further

understanding of what genes and chromosomal regions underlie traits under selection in the hatchery environment.

5. Conclusion

Overall, results presented here demonstrate that the number of broodstock with high reproductive success is the major determinant of the genetic diversity of stocked fingerlings as reproductive success is highly variable and only a small proportion of broodstock are successful at each spawning event. This underscores the importance of regularly rotating and exchanging broodstock. Next to family effects, our results demonstrate that rearing progeny in outdoor ponds throughout the summer without strictly controlled conditions has a significant, albeit smaller, effect on the genetic diversity of stocked fingerlings and therefore nmay ensure that fish released into the wild populations still harbor the ability to tolerate a range of conditions and counteract potential domestication effects (Belk et al., 2008; Rasmussen et al., 2009; Tave and Hutson, 2019). Higher rates of post-stocking survival for fish reared in semi-natural ponds relative to traditional hatchery methods such as tanks and raceways have been observed in a range of species (Fuss and Byrne, 2002; McKeown et al., 1999; Rasmussen et al., 2009; Tipping, 2001). This is likely because while rearing progeny in a hatchery setting gives managers more control over survival, it may have unintended genetic, morphological, physiological, and behavioral consequences that ultimately reduce viability in the wild (Le Vay et al., 2007; Rasmussen et al., 2009; Ryman and Laikre, 1991). With hatchery-based stock augmentation becoming an increasingly important fisheries management tool, strategies must be re-evaluated to reduce the risk of producing fish that are genetically impoverished or behaviorally compromised (Bordeleau et al., 2018; Fernö et al., 2006; Molnár et al., 2018). This will likely need to include a shift away from raceways and tanks used at a commercial hatchery to maximize production, towards increasingly relying on semi-natural environments such as outdoor ponds or purpose-built natural environments, including cages/enclosures in lakes and coastal areas (Feuerbacher et al., 2016; Tave and Hutson, 2019).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2022.738539.

CRediT authorship contribution statement

Shannon J. O'Leary – Conceptualization, Project administration, Formal analysis, Visualization, Writing – original draft, Writing review & editing, Christopher M. Hollenbeck – Formal analysis, Writing – review & editing, Robert R. Vega, - Resources, Writing – review & editing, Ashley Fincannon - Resources, Writing – review & editing, David S. Portnoy – Conceptualization, Analysis, Funding acquisition, Writing – review & editing.

Data availability statement

Raw, demultiplexed sequence reads are available from NCBI's Short Read Archive, and the filtered SNP data set (vcf format) is available from the authors upon request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Raw, demultiplexed sequence reads are available from NCBI's Short Read Archive, and the filtered SNP data set (vcf format) is available from the authors upon request.

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